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REPORT REFERENCE: 135

TITLE: Genetic Evaluation of Dow Corning Q7-2159A Medical Gel in the In Vitro Mammalian Cell Transformation Assay. Dow Corning Tox. File No. 2476-7

Date: August 8, 1986

AUTHORS: A.J. Isquith and R.T. Henrich

SOURCE: Internal Dow Corning study

MATERIAL: Silicone mammary gel

GLP STATUS: Yes

ABSTRACT: A tissue culture medium extract of Q7-2159A was evaluated for an ability to induce morphologic transformation of BALB/C-3T3 cells in culture with and without metabolic activation. No activity was found.

ADVERSE EFFECTS: None

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DOW CORNING CORPORATION
- Toxicology Department

PROPRIETA

File No.: 2476-7

GENETIC EVALUATION OF DOW CORNING® Q7-2159A* IN THE IN VITRO MAMMALIAN CELL TRANSFORMATION ASSAY

Reference No.: TX-85-7000-01

Series No.: I-0005-1536

Reference No.:

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Submitted By: Regina M. Malczewski

Date: August 8, 1986

This summary of data and conclusions is based upon the sample received. Additional studies may be required as specific uses and formulations are developed or if process changes occur.

ABSTRACT

The test material was evaluated for its ability to induce morphological transformation of BALB/C-3T3 cells in culture both with and without metabolic activation. The material was found to be inactive in the induction of morphologically transformed cells.

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*DOW CORNING® Q7-2167/DOW CORNING® Q7-2168 Medical Gel

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TABLES -

OBJECTIVE

The objective of this assay is to evaluate the test article for its ability to induce morphological transformation of cultured BALB/C-3T3 cells. Transformation of BALB/C-3T3 cells is recognized by the appearance of dense, piled-up foci of altered cells superimposed on a monolayer of normal cells.

RATIONALE

BALB/C-3T3 mouse cells will multiply in culture until a monylayer is achieved and will then cease further division. These cells, if injected into immunosuppressed, syngeneic host animals, will not produce neoplastic tumors. However, cells treated in vitro with chemical carcinogens will give rise to foci of cellular growth superimposed on the cell monolayer. If these foci are picked from the cultures, grown to larger numbers and injected into animals, a malignant tumor will in most cases be obtained. Thus, the appearance of foci of altered cells is correlated with malignant transformation.

MATERIALS

1. Indicator Cells

Clone 1931 of BALB/C-3T3 mouse cells was obtained from American Type Culture Collection (ATCC). Further subclones, selected for low spontaneous frequencies of foci formation, are used for assays. Stocks are maintained in liquid nitrogen and laboratory cultures are checked periodically to ensure the absence of mycoplasma contamination. Cultures are grown and passaged weekly in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% calf serum (CS). They were maintained at 37° ± 2°C in a humidified atmosphere containing approximately 5% CO_2.

2. Control Articles

A) Solvent/Negative Controls

A solvent control was performed for each portion of the assays by carrying cells unexposed to the test material through all of the assay procedures. In the activation portion of the assay, the solvent control cultures were exposed to the S-9 activation mix. The solvent used in this assay was Eagle's Minimum Essential Media (EMEM).

B) Positive Controls

3-Methylcholanthrene (3-MCA) was used as a positive control in the non-activation assay. This chemical was used at a final concentration of 5 µg/ml.

Benzo(a)pyrene (BP) was used as a positive control in the activation assay. This chemical was used at a final concentration of 12.5 µg/ml.

EXPERIMENTAL DESIGN

1. Preliminary Cytotoxicity Testing

Since the material to be tested was a gel, which was not soluble in any solvent compatible with the biological test system, an extraction procedure was used. One gram of the test material was extracted with 10 ml Eagle's Minimum Essential Medium (EMEM) on a New Brunswick rotary shaker for 24 hours/37°C/150 rpm. Twelve dose levels were chosen to determine the dose range to be employed in the transformation assay. The growth medium used was EMEM with 10% cs.

Cells were seeded at 200-250 cells/60 mm dish and were cultured for 24 hours in 5 ml of growth medium. The cells were then exposed, in both the presence and absence of S9 activation, to each dose. The cells being exposed in the absence of metabolic activation were given a three-day exposure period and those in the presence of metabolic activation a four-hour exposure period. After either a three-day or four-hour exposure period, the cells were washed and incubated in fresh growth medium for an additional 7-10 days. The surviving colonies were stained and counted. A relative survival for each dose was obtained by comparing the number of colonies surviving treatment to the colony counts in the solvent control dishes. The highest dose chosen for the subsequent transformation assay would normally have caused no more than a 80-90% reduction in the colony-forming ability of the 3T3 cells (Rundell, J. O., et al., 1983). Since none of the doses caused a 80-90% reduction in the colony-forming ability of the 3T3 cells, the maximum dose used in both transformation assays was 2000 µg/ml. Two lower doses were also selected for the transformation assays.

2. Transformation Assay

A) Non-activation Assay

The procedure used was adopted from that reported by Kakunaga (1973). Exponentially growing BALB/C-3T3 cells are seeded at 200-250 cells/60 mm dish for the cytotoxicity studies of each treatment and at 1 x 10^4 cells/60 mm dish in 15 replicate dishes per condition for the transformation assay. After a 24-hour incubation, the dishes were treated for each of the following conditions: three preselected doses of the test material; positive control; and solvent control. All testing was carried out in 5 ml of growth medium. The dishes were incubated for three days in the presence of the test material at 37°C. After the exposure period, the medium was removed and the dishes were washed with Hank's Balanced Salt Solution (HBSS). Fresh growth media was

added and the dishes were reincubated for approximately 8-10 days for the cytotoxicity study and four weeks for the transformation assay. During this time, the media was changed twice a week.

At the end of the respective incubation periods, all colonies were fixed with methanol and stained with Giemsa. The stained dishes were examined by eye with a microscope to determine the number of surviving colonies for the cytotoxicity assay and the number of foci of transformed cells for the transformation assay.

B) Activation Assay

The activation assay was performed independently with its own set of solvent and positive controls. The procedure was identical to the nonactivation assay except for the addition of the S-9 fraction of rat liver homogenate, Aroclor 1254 induced, obtained commercially from Hazleton Laboratories (formerly Litton Bionetics, Incorporated), Kensington, Maryland, and necessary cofactors during the four-hour treatment period.

RESULTS AND DISCUSSION

The results are presented in Table I-IV. In the presence of metabolic activation (Table I), the test material showed no toxicity at any of the dose levels and the plating efficiency remained high. The same was true in the absence of metabolic activation as can be seen in Table II.

Tables III and IV represent the results of the transformation assays with and without metabolic activation, respectively. No significant increase in the transformation frequency was observed either with or without activation. The test material should be considered inactive in inducing morphological transformation of BALB/C3T3 cells.

DATA PRESENTATION

1. Relative Survival

Relative Survival (%) = (Average number of colonies per treated culture/ average number of colonies per solvent control dish) x 100%.

2. Plating Efficiency (PE)

Plating Efficiency (%) = (Average number of colonies per dish/number of cells seeded) x 100%.

3. Cell at Risk (CAR)

CAR = Number of dishes x 1 x 10^4 x PE.

4. Transforming Frequency (TF)

TF = Number of foci/CAR.

STATISTICAL EVALUATION

Statistical tables from Kastenbaum and Bowman (1970) were utilized to determine the statistical significance at each dose level versus the negative control at the 95% or 99% confidence level. The 95% confidence level is the minimum acceptable level for considering the test material to be positive for transforming activity.

REFERENCES

1. Kakunaga, T., 1973. A quantitative system for assay of malignant transformation by chemical carcinogens using a clone derived from BALB/3T3. Int. J. Cancer 12:463-473.

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4. Schechtman, L. M. and Kouri, R. E., 1977. Control of benzo(a)-pyrene-induced mammalian cell cytotoxicity, mutagenesis and transformation by exogenous enzyme fractions. In: Progress in Genetic Toxicology, D. Scott, B. A. Bridges and F. E. Sobels, eds. Elsevier/North-Holland Biomedical Press, New York, pp. 307-316.

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This report constituted of pages 1-8, and Tables I-IV, signed this 8th day of August, 1986.

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QUALITY ASSURANCE STATEMENT

This report represents data generated by the Toxicology Department, Dow Corning Corporation, Midland, Michigan. This study was conducted according to EPA Toxic Substances Control; Good Laboratory Practices Regulations; 40 CFR, Part 797, Vol. 48, No. 230. The results reported accurately reflect the data generated. All raw data is located at Dow Corning Corporation.

Study Started:	January 27, 1986
Study Completed:	March 31, 1986
Date Audited:	January 27, 1986 and March 31, 1986
Report Issued:	August 8, 1986

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August 8, 1986
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TABLE I

Cytotoxicity Assay with Metabolic Activation

Treatment	Final Concentration (ug/ml)	Plating Efficiency (%)	Relative Survival (%)
Solvent Control			
EMEM[a]		81.2	100.0
Positive Control			
BP[b]	12.5	75.6	93.2
Test Material			
TX-86-7000-01	2000	78.2	96.3
	1000	82.4	101.5
	500	79.4	97.8

[a] EMEM = Eagle's Minimum Essential Medium

[b] BP = Benzo(a)pyrene

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TABLE II

Cytotoxicity Assay Without Metabolic Activation

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Treatment	Final Concentration (ug/ml)	Plating Efficiency (%)	Relative Survival (%)
Solvent Control			
EMEM[a]	-	77.4	100.0
Positive Control			
3-MCA	5.0	70.4	91.0
Test Material			
TX-86-7000-01	2000	82.2	106.2
	1000	84.4	109.1
	500	75.4	97.4

[a] EMEM = Eagle's Minimum Essential Medium

[b] 3-MCA = 3-Methylcholanthrene

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TABLE III

Transformation Assay in the Presence of Exogenous Metabolic Activation

Treatment	Final Concentration (µg/ml)	CAR ($\times 10^3$)	Foci	TF ($\times 10^{-4}$)
Solvent Control				
EMEM[a]	–	121.8	1	0.08
Positive Control				
BP[b]	12.5	113.4	12	1.06*
Test Material				
TX-86-7000-01	2000	117.3	1	0.09
	1000	123.6	0	0.00
	500	118.9	2	0.17

[a] EMEM = Eagle's Minimum Essential Medium

[b] BP = Benzo(a)pyrene

*Significant increase, $p<0.05$

TABLE IV

Transformation Assay in the Absence of Exogenous Metabolic Activation

Treatment	Final Concentration (ug/ml)	CAR ($\times 10^3$)	Foci	TF ($\times 10^{-4}$)
Solvent Control				
EMEM[a]	-	115.9	2	0.17
Positive Control				
3-MCA[b]	5.0	121.8	14	1.15*
Test Material				
TX-86-7000-01	2000	123.3	2	0.16
	1000	125.6	1	0.08
	500	112.9	1	0.09

[a] EMEM = Eagle's Minimum Essential Medium

[b] 3-MCA = 3-Methylcholanthrene

*Significant increase, $p<0.05$